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X-Y Sperm Aneuploidy in 2 Cattle *(Bos taurus)* Breeds as Determined by Dual Color Fluorescent in situ Hybridization (FISH)

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Abstract

The present study was undertaken to investigate aneuploidy rates in the sperm populations of 2 cattle *(Bos taurus)* breeds by using dual color fluorescent in situ hybridization (FISH) with Xcen and Y chromosome-specific painting probes, obtained by chromosome microdissection and DOPPCR. Frozen semen from 10 Italian Friesian and 10 Italian Brown testing bulls was used for the investigation. For each bull, more than 5,000 sperm were analyzed, for a total of 52,586 and 51,342 sperm cells for the 2 breeds, respectively. The present study revealed – in both breeds – a preponderance of the Y-bearing sperm compared to the X-bearing sperm. Within each breed, a statistically significant variation in the various classes of aneuploidy (XX, YY and XY) was found: differences were found in the Friesian breed among the 3 diploidy classes, and in the Brown breed, among the 3 disomy classes (p ! 0.05) as well as among the 3 diploidy classes (p ! 0.01). However, the 2 breeds did not differ significantly in the overall mean rates of X-Y aneuploidy (disomy + diploidy) which amounts to 0.162% in the Italian Friesian and 0.142% in the Italian Brown. When meiosis I (MI) and II (MII) errors were compared, statistically significant differences (p ! 0.01) were found in the disomy classes and in both breeds, whereas the differences between diploidy classes were not significant. Compared to humans, a lower level of aneuploidy has been found in the domestic species analysed so far. The present study contributes to the establishment of a baseline level of aneuploidy in the sperm populations of 2 cattle breeds which could be used for monitoring future trends of reproductive health, especially in relation to environmental changes and mutagens.

Introduction

Sperm chromosomes of domestic animals have been investigated by conventional cytogenetic methods since 1987 [Creighton and Houghton, 1987; Tateno and Mikamo, 1987] by using a quite expensive and laborious ‘pig or cattle/hamster’ heterospecific in vitro fertilization system.

The first report on the application of FISH directly on pig sperm was published by Kawarasaki et al. [1995], by using a male specific DNA probe; other contributions were made on pig, by using

molecular probes for chromosomes Y and 1 [Kawarasaki et al., 1996; Parrilla et al., 2003], or flow-sorted chromosomes X-Y [Kawarasaki et al., 1998], and on cattle, by using molecular probes for chromosome Y [Kobayashi et al., 1999], cosmid PL44 for the X and painting probe for the Y [Hassanane et al., 1999] or BACs for chromosome X and the repetitive sequence BRY4a for the Y [Piumi et al., 2001]. Chromosome-specific painting probes produced by chromosome microdissection or chromosome sorting followed by DOP-PCR were also used in the pig [Rubes et al., 1999] as well as in cattle [Rens et al., 2001; Révay et al., 2002] and in cattle, river buffalo, sheep and goat [Di Berardino et al., 2004]. Recently, Bonnet-Garnier et al. [2006] used bovine chromosome-specific painting probes for chromosomes and 29 to study meiotic segregation of the translocated chromosomes in sperm.

While the majority of these papers demonstrated the usefulness of the FISH technique as a method for validating sperm sexing technologies, only a few of them investigated aneuploidy rates. Since chromosomal abnormalities in the germ cells are one of the most important causes of embryonic mortality [King, 1990], and since we do not have sufficient information about the real impact of aneuploidy in the various domestic species and breeds, we aimed to further, at least partially, this knowledge.

Material and Methods

Chromosome Microdissection and Probe Preparation

Metaphase cells for the production of probes ‘via’ microdissection were prepared according to standard cytogenetic techniques [Iannuzzi and Di Berardino, 2008]. For microdissection, the fixed lymphocyte suspension was spread onto a precleaned 24 ! 60-mm coverslip which was then air dried and treated for GTG-banding.

The Xcen probe was produced by isolating the pericentromeric region, corresponding to the centromere and to the region Xp14–p11 of the standardized GTG-banded karyotype; the Y probe was produced by scraping the whole chromosome. Microdissected chromosomes were amplified

following the protocol of Engelen et al. [1998]. Probes were labeled with dUTP-11-digoxigenin (Xcen) and dUTP-16-biotin (Y) (both from Roche), respectively, in a second DOP-PCR reaction, using 2 _ l of product from the first reactions as template.

Semen Samples

Cryopreserved semen samples belonging to the Italian Friesian and Italian Brown breeds of cattle were obtained from artificial insemination centers and private farms. For each breed, frozen semen from 10 animals was used for this study. All animals showed normal semen parameters and were all karyologically normal (2n = 60)

In situ Hybridization

The Xcen and Y probes were hybridized simultaneously on metaphase plates for validation and subsequently on decondensed sperm. Decondensation of sperm nuclei was performed following the method described by Han et al. [1992]. Probes were precipitate in the presence of 10 µ g of salmon sperm DNA and 10 µ g of calf thymus DNA (both from Sigma), dissolved in 15 µ l of hybridization solution (50% formamide in 2 ! SSC + 10% dextran sulphate; both from Sigma), denatured at 72 ° C for 10 min and incubated at 37 ° C for 90 min. Metaphase and sperm preparations were denatured for 2 and 5 min respectively in a solution of 70% formamide in 2 ! SSC (pH 7.0) at 72 ° C. The slides were hybridized in a moist chamber at 37 ° C overnight. After hybridization and slide washing, the biotin-labeled probe was revealed using a green Alexa 488 fluorochrome conjugated to streptavidin (Invitrogen), and the digoxigenin-labeled probe using a red Rhodamine fluorochrome conjugated to an anti-digoxigenin antibody from sheep (Roche). Slides were counterstained with DAPI (0.24 µg/ml; Sigma) in Antifade (Vector Lab).

Fluorescence Analysis and Scoring

The slides were observed at 100 ! magnification with a Leica DMRA fluorescence microscope equipped with DAPI, FITC, Cy3 specific filters, the DAPI/FITC/TXRD triple filter, and phase-contrast optics. Digital images were captured using the Leica QFISH software. At least 5,000 sperm nuclei were examined for each animal. The scoring was carried out using strict scoring criteria [Robbins et al., 1995]. Overlapped cells and those with ambiguous FISH signals were not scored. For each nucleus, type (X or Y) and number of sex chromosomes were analyzed. Sperm with one signal (green or red) were scored as normal haploid; spermatozoa with 2 signals were classified as disomic (XX, YY and XY depending on the 2 signal colors). Diploid sperm were distinguished from disomic sperm on the basis of their size [Joseph et al., 1984]. Since sperm decondensation might not be uniform along the slide, size comparison was made only within the same microscopic field where the diploid sperm were found. In any case, phase-contrast optics was used to check for the presence of the tail. Furthermore, sperm nuclei without signal were scored to calculate hybridization efficiency.

Validation of the Data

In the present study, disomies and diploidies were differentiated according to the size of the nuclei; to verify if this could lead to errors in the estimation of aneuploidy, we performed an additional hybridization experiment on a limited sample of 6 animals (3 for each breed, previously analyzed with Xcen and Y probes) by using a probe for chromosome 6 [Habermann et al., 2005], labeled in red, and the 2 probes for X-Y chromosomes, both labeled in green. Hybridization conditions were the same as above. Ten thousand sperm were scored for each subject, in order to calculate the frequencies of diploidy (without distinguishing between XX, XY and YY diploidy), disomy for sex chromosome (without distinguishing between XX, XY and YY disomy) and disomy for chromosome 6. The results were then compared with those obtained on the same subjects using only Xcen and Y probes.

Statistical Analysis

The following statistics were used: the _2 test with Yates' corrections for interindividual differences; the Kruskal-Wallis and the Mann-Whitney tests were used for multiple comparisons and for class differences.

Results

Figure 1 shows the Xcen and Y painting probes, DAPI staining and their diagrammatic representation. Figure 2 shows disomic and diploid XX, XY and YY sperm after FISH staining.

Interindividual Variations within the Breed (table 1)

Totally, more than 100,000 sperm were examined with Xcen and Y probes, and more than 50,000 sperm analysed for each breed (10 bulls). The hybridization efficiency was high, around 98–99% in all cases, except for subject 5 of the Friesian breed, where the hybridization efficiency was 92.34%. A deviation from the expected 1: 1 ratio between the X- and Y-bearing sperm was found in both breeds in favour of the Y chromosome (p ! 0.05); such a deviation was particularly evident on subject 8 of the Friesian breed. In the Friesian breed the differences among individuals for each class of aneuploidy (XX, XY, YY) were not statistically significant. In the Brown breed, differences among the animals were again not significant, except for the XY disomy ($p < 0.05$).

XX, YY, XY Class Comparison within the Breed (table 2)

In the Friesian breed, statistically significant differences ($p < 0.05$) were found only among the diploidy classes, whose frequencies were 0.015%–0.006%–0.029%, whereas in the Brown breed, differences were found not only among the 3 diploidy classes (0.018%–0.004%–0.041%) ($p < 0.01$) but also among the 3 disomy ones (0.022%–0.045%–0.012%) ($p < 0.05$).

To analyze possible differences in the occurrence of errors during meiosis I (XY disomic/diploid sperm) or meiosis II (XX-YY disomic/diploid sperm) we applied the Mann-Whitney test. Meiotic errors giving rise to disomies were significantly (p ! 0.01) more frequent in MII than in MI (0.081%

vs. 0.031% in the Friesian, 0.067% vs.0.012% in the Brown). Concerning the diploidy, the differences between MI and MII meiotic errors were not statistically significant. The overall frequency (disomies + diploidies) of errors in MII was higher than the frequency of errors in MI in both breeds ($p < 0.05$).

Interbreed Comparison (table 3)

The 2 breeds analyzed were basically similar in the overall frequency of X-Y chromosome aneuploidy (0.162% vs. 0.142%, respectively for Friesian and Brown). However, while in the Friesian breed the disomies (0.112%) were more frequent than diploidies (0.050%) (p ! 0.01), in the Brown the 2 types of abnormalities were basically similar (0.079% and 0.063%, respectively). The different classes of diploidy were equally represented between the 2 breeds; differences (p ! 0.05) were found at the level of XX disomy (0.044% vs. 0.022%) and XY disomy (0.031% vs. 0.012%).

Validation of the Data and Disomy of Chromosome 6 (table 4 , fig. 3)

The analysis of data from the second set of slides on 6 subjects revealed a substantial similarity in the frequencies of diploidy (0.029% vs. 0.025%) and sex chromosomes disomy (0.139% vs. 0.142%) detected using the 2 different sets of probes. Disomy for chromosome 6 had an average frequency of 0.080% (range 0.038–0.116%).

Discussion

The results of the present study indicated that in both breeds there was a significant preponderance of the Y-bearing sperm compared to the X. This finding is similar to that previously reported by Hassanane et al. [1999] on the Swedish Friesian breed. Unfortunately, due to the paucity of studies on this topic, we do not know whether this finding is a sporadic occurrence or a consistent phenomenon which is ongoing in the Friesian breed, but certainly cannot be explained as due to the low intensity of any of the 2 signals, as could happen when using small-size cosmid or BACs. In the present case, in fact, the probes we used provide quite strong signals being of the ‘painting’ type. It

is therefore necessary to expand such investigations in order to provide more information on this aspect. The present study also indicated a quite low interindividual variability in the frequencies of the different aneuploidy classes. Since the 2 breeds analysed in the present study are highly selected, the consequent great genetic uniformity could explain the low variability observed. The analysis of unselected breeds (or genetic types) would clarify if this low variability is characteristic of the species *Bos taurus* or is a consequence of the genetic uniformity due to selection. The analysis 'within' each breed showed several differences. In the Italian Friesian, disomies were more frequent than diploidies, but while the former were equally represented in the 3 classes, for the latter XY diploidies showed a higher frequency compared to YY. On the contrary, in the Brown breed, disomies and diploidies had the same frequencies in both cases, while the frequencies of the different classes were different. In this breed, disomic YY sperm had a higher frequency compared to XY disomic sperm. Also in this breed, like in the Italian Friesian, XY diploidies were the most represented. In order to explain this difference, abnormalities were classified according to their origin as arising from errors in meiosis I (XY disomic/diploid sperm) or meiosis II (XX-YY disomic/diploid sperm). Disomies were more frequent due to errors occurring during MII in both breeds, while diploidies originated from errors in MI or in MII with the same frequencies. This means that diploidy can originate with the same frequencies as a consequence of chromatid or chromosomal nondisjunction events, while, at least for sex chromosomes, disomies originate mainly from chromatid nondisjunction.

Usually, the reduced pairing of sex chromosomes during MI is considered responsible for their higher aneuploidy incidence compared to autosomes [Shi and Martin, 2000]. However, in humans, while some authors confirmed this finding [Robbins et al., 1995; Downie et al., 1997], others [Spriggs et al., 1995; Rubes et al., 2005] did not find differences in the frequencies of MI and MII errors, thus indicating that other factors could be responsible for the higher incidence of sex chromosome aneuploidy in sperm. Our results seem to confirm the latter hypothesis, showing that X and Y disomies originate mainly from chromatid nondisjunction, indicating that, in this species, the reduced pairing of X and Y is responsible for only a small part of the total disomies. The interbreed

comparison (table 3) showed that the 2 breeds did not differ from each other in the overall rate of X and Y aberrant spermatozoa (disomic + diploid) which were 0.162% in the Friesian and 0.142% in the Brown. The diploidy rate was very similar (0.050% vs. 0.062%), while for the disomy rate Italian Friesian showed a higher frequency than Italian Brown (0.112% vs. 0.079%), but this difference was not significant for p = 0.05. Due to the paucity of scientific reports on aneuploidy rates in sperm of cattle, we could compare the present results only to those previously reported by Hassanane et al. [1999] and by Di Berardino et al. [2004] (table 3). Concerning the Italian Friesian breed, close similarities can be noticed between the results of the present study and those previously reported by Hassanane et al. [1999] in the Swedish Friesian breed: in fact, the X-Y disomy rates are 0.112% and 0.125%, respectively, whereas the X-Y diploidy rates are 0.050% and 0.045%, respectively. Such agreement in the results can be further explained by the fact that the Friesian breed of cattle is highly selected everywhere, around the world, and the connections between countries could have led to a homogenization of the population from a genetic point of view. From table 3 it can also be seen that the aneuploidy rates detected in cattle breeds, so far, are also comparable to those reported in several other breeds of pig [Rubes et al., 1999]. The YY disomy rates in cattle, in fact, vary between 0.029% and 0.045%, quite close to the value observed in the Bohemian Fleshy breed (0.058%), being lower than those found in the Large White (0.112%), White Improved (0.125%) and Pietrain (0.170%), but higher than those of the Landrace (0.006%) and Duroc (0.009%). Surprisingly, the YY diploidy rate found in cattle is between 0.004% and 0.024%, much less compared to the corresponding values observed in the pig breeds, where the YY diploidy rate varies from 0.133% to 0.350%.

In order to compare cattle with other domestic species, we averaged the mean aneuploidy values previously reported in the Swedish Friesian [Hassanane et al., 1999] with those reported in the present study on the Italian Friesian and Italian Brown breeds (table 5). The mean rates for cattle *(Bos taurus)* resulted in 0.105% for disomy, 0.052% for diploidy and 0.157% for the total aneuploidy rate. These values were then compared to those of the river buffalo (*Bubalus bubalis* , river type), sheep *(Ovis aries)* and goat *(Capra hircus)* reported by Di Berardino et al. [2004], pig *(Sus scrofa*

domestica) reported by Rubes et al. [1999], mouse *(Mus musculus)* reported by Adler et al. [1996] and humans *(Homo sapiens)* calculated as average values [Griffin et al., 1995; Martin et al., 1995; Robbins et al., 1995; Spriggs et al., 1995; Downie et al., 1997; Rubes et al., 2005]. By taking human as reference: (a) the total disomy rates found in the domestic species investigated, so far, were all lower, being comprised of 0.080% (mouse) to 0.164% (goat); (b) the total diploidy rates were higher only in the goat (0.229%) and lower in the others, being comprised of 0.033% (sheep) and 0.177% (pig); (c) the total aneuploidy (disomy + diploidy) rates were all lower, being comprised of 0.033% (sheep) to 0.393% (goat). The fact that in the domestic species the aneuploidy rates are lower compared to human could, at least in part, be explained by the fact that domestic animals have been strongly selected for fertility, and this could have led to a reduction in the frequencies of aberrant spermatozoa. To verify this hypothesis, it could be useful to investigate wild species belonging to the same family as the domestic ones, in order to clarify the effects of the artificial selection on the sperm aneuploidy rate. In addition, domestic animals have been targeted by FISH only recently and, therefore, only few data are available, whereas in humans such studies are more abundant and consistent. The present work focused on X and Y sperm aneuploidy, using sperm size as a criteria for distinguishing between disomic and diploid sperm. Size comparison was made only within the same microscopic field where the diploid sperm were found and phase-contrast optics was used to check for the presence of the tail, to be certain that it was not a round cell. To verify if this kind of approach could determine an 'under' or an 'over' estimation in the frequencies of the different classes of abnormalities, we performed an additional experiment by using an autosomal probe (chromosome 6) on a limited number of subjects.

As shown in table 4 , the data obtained from the 2 analyses were very similar, thus indicating that, at least under the experimental conditions used in the present study, the scoring criteria we used were sufficient to distinguish between disomic and diploid cells. In addition, disomy of chromosome 6 had an incidence of 0.080%, lower than disomy of the X-Y chromosomes in the animals tested, except for one. As in humans, it seems that the sex chromosomes are more prone to undergo non-disjunction

events. However, it will be necessary to extend the analysis to a higher number of animals and to use more probes, in order to test possible interchromosomal differences.

Since mammalian fertility is strongly affected by chromosomal abnormalities, which are responsible for nearly 70% of the embryonic mortality in humans [Hassold, 1998] as well as in domestic animals [King, 1990; Vanroose et al., 2000], further studies of germ cells (sperm and oocytes) should be implemented in order to better understand the genetic causes of aneuploidies and their impact on the reproductive and productive efficiency of domestic animals. Several studies [for a review, see Pacchierotti et al., 2007] showed that chemical substances, commonly used in medicine and agriculture (drug and pesticide) or present in the environment as pollutant, can increase the frequencies of germ cell aneuploidy in human and mouse. Domestic animals are often exposed to such substances through the farming environment or feedstuff; and the sperm-FISH assay can represent a useful tool to allow the identification of chemicals that can negatively affect the animal's health, thus reducing its reproductive efficiency.

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Fig. 1. Xcen and Y painting probes, DAPI staining and diagrammatic representation

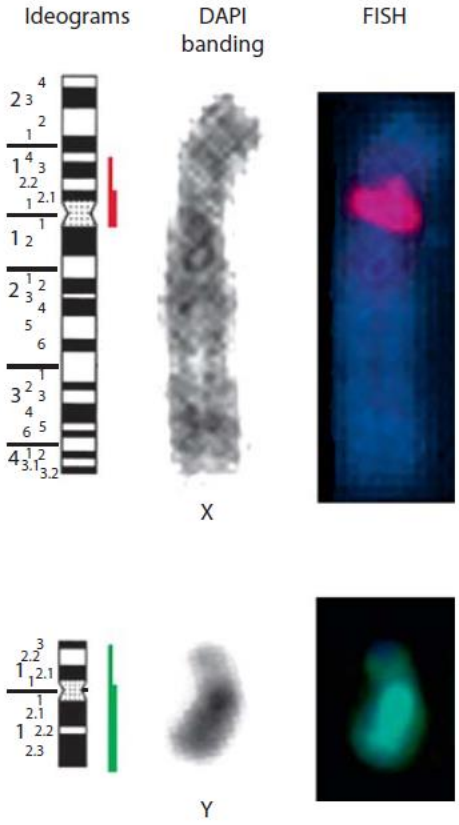

Fig. 2. Fluorescent in situ hybridization (FISH) on cattle sperm showing XX, XY and YY disomic and diploid sperm.

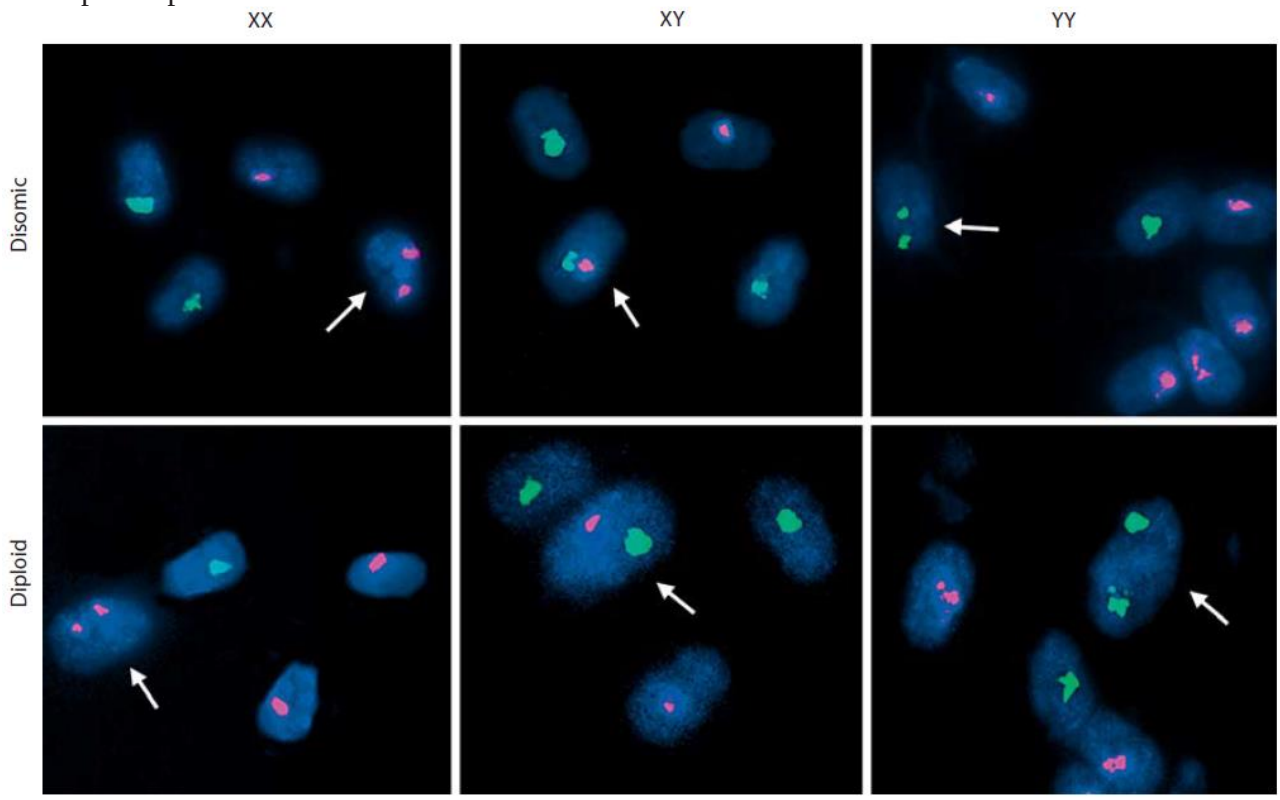

Fig. 3. Fluorescent in situ hybridization (FISH) on cattle sperm using X-Y probe (green) and chromosome 6 probe (red) showing disomic (**a**) and diploid (**b**) sperm.

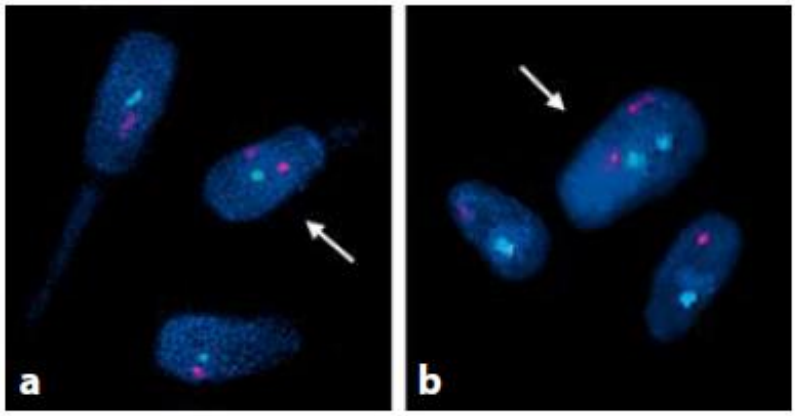

Table 1. Number and frequency (%) of X- and Y-bearing, disomic and diploid sperm in bulls of the Italian Friesian (I.F.) and Italian Brown (I.B.) breeds of cattle

	X	Y	Disomic			Diploid			With signal	Without signal	Total sperm
			XX	YY	XY	XX	YY	XY			
I.F.											
1	2,819 (46.968)	3,154 (52.549)	1 (0.017)	5 (0.083)	1 (0.017)	1 (0.017)	0 (0)	0 (0)	5,981 (99.650)	21 (0.350)	6,002
2	2,411 (47.081)	2,623 (51.220)	4 (0.078)	5 (0.098)	2 (0.039)	1 (0.020)	0 (0)	0 (0)	5,046 (98.535)	75 (1.465)	5,121
3	2,643 (51.300)	2,487 (48.273)	5 (0.097)	2 (0.039)	2 (0.039)	1 (0.019)	0 (0)	1 (0.019)	5,141 (99.786)	11 (0.214)	5,152
4	2,509 (49.380)	2,564 (50.463)	2 (0.039)	0 (0)	1 (0.020)	0 (0)	0 (0)	0 (0)	5,076 (99.902)	5 (0.098)	5,081
5	2,420 (44.658)	2,574 (47.500)	2 (0.037)	1 (0.018)	2 (0.037)	1 (0.018)	0 (0)	4 (0.074)	5,004 (92.342)	415 (7.658)	5,419
6	2,257 (47.080)	2,468 (51.481)	0 (0)	2 (0.042)	1 (0.021)	1 (0.021)	1 (0.021)	1 (0.021)	4,731 (98.686)	63 (1.314)	4,794
7	2,510 (47.225)	2,761 (51.947)	2 (0.038)	0 (0)	1 (0.019)	0 (0)	1 (0.019)	2 (0.038)	5,277 (99.285)	38 (0.715)	5,315
8	2,380 (44.846)	2,878 (54.230)	4 (0.075)	2 (0.038)	2 (0.038)	0 (0)	1 (0.019)	2 (0.038)	5,269 (99.284)	38 (0.716)	5,307
9	2,472 (46.094)	2,854 (53.216)	3 (0.056)	1 (0.019)	2 (0.037)	1 (0.019)	0 (0)	2 (0.037)	5,335 (99.478)	28 (0.522)	5,363
10	2,372 (47.138)	2,645 (52.564)	0 (0)	1 (0.020)	2 (0.040)	2 (0.040)	0 (0)	3 (0.060)	5,025 (99.861)	7 (0.139)	5,032
All	24,793 (47.148)	27,008 (51.360)	23 (0.044)	19 (0.037)	16 (0.031)	8 (0.015)	3 (0.006)	15 (0.029)	51,885 (98.667)	701 (1.333)	52,586
I.B.											
1	2,548 (49.824)	2,505 (48.983)	2 (0.039)	4 (0.078)	0 (0)	1 (0.020)	0 (0)	1 (0.020)	5,061 (98.964)	53 (1.036)	5.114
2	2,474 (47.559)	2,669 (51.307)	0 (0)	3 (0.058)	0 (0)	1 (0.019)	0 (0)	2 (0.038)	5,149 (98.981)	53 (1.019)	5,202
3	2,444 (47.383)	2,632 (51.028)	0 (0)	6 (0.116)	2 (0.039)	0 (0)	0 (0)	0 (0)	5,084 (98.565)	74 (1.435)	5,158
4	2,635 (49.905)	2,589 (49.034)	2 (0.038)	1 (0.019)	0 (0)	2 (0.038)	0 (0)	3 (0.057)	5,232 (99.091)	48 (0.909)	5,280
5	2,505 (48.802)	2,576 (50.185)	2 (0.039)	1 (0.019)	0 (0)	2 (0.039)	0 (0)	2 (0.039)	5,088 (99.123)	45 (0.877)	5,133
6	2,374 (45.866)	2,714 (52.434)	1 (0.019)	2 (0.039)	0 (0)	0 (0)	0 (0)	2 (0.039)	5,093 (98.396)	83 (1.604)	5,176
7	2,246 (44.643)	2,715 (53.965)	3 (0.060)	1 (0.020)	0 (0)	0 (0)	0 (0)	6 (0.119)	4,971 (98.807)	60 (1.193)	5,031
8	2,327 (46.318)	2,664 (53.025)	1 (0.020)	3 (0.060)	1 (0.020)	0 (0)	1 (0.020)	1 (0.020)	4,998 (99.482)	26 (0.518)	5,024
9	2,345 (45.209)	2,796 (53.904)	0 (0)	1 (0.019)	0 (0)	1 (0.019)	1 (0.019)	1 (0.019)	5,145 (99.190)	42 (0.810)	5,187
10	2,415 (47.945)	2,590 (51.419)	0 (0)	1 (0.020)	3 (0.060)	2 (0.040)	0 (0)	3 (0.060)	5,014 (99.543)	23 (0.457)	5,037
All	24,313 (47.355)	26,450 (51.517)	11 (0.022)	23 (0.045)	6 (0.012)	9 (0.018)	2 (0.004)	21 (0.041)	50,835 (99.013)	507 (0.987)	51,342

Table 2. Statistical signifi cance of the comparisons 'within' each breed in the frequency of the different aneuploidy classes

	Italian Friesian			Italian Brown		
	%	com-parison	p	%	com-parison	p
Disomy						
XX (1)	0.044	1–2	NS	0.022	1–2	NS
YY (2)	0.037	2–3	NS	0.045	2–3	<0.05
XY (3)	0.031	1–3	NS	0.012	1–3	NS
Tot (4)	0.112	4–10	<0.01	0.079	4–10	NS
MI (5)	0.031	5–6	<0.01	0.012	5–6	<0.01
MII (6)	0.081	–		0.067	–	
Diploidy						
XX (7)	0.015	7–8	NS	0.018	7–8	NS
YY (8)	0.006	8–9	<0.05	0.004	8–9	<0.01
XY (9)	0.029	7–9	NS	0.041	7–9	NS
Tot (10)	0.050	–		0.063	–	
MI (11)	0.029	11–12	NS	0.041	11–12	NS
MII (12)	0.021			0.022		

Table 3. Interbreed variability in the frequency of X-Y sperm aneuploidy (disomy + diploidy)

Trait	Cattle			Pig					
	S.F.	I.F.	I.B.	L.W.	B.F.	L.	W.I.	D.	P.
No. animals	5	10	10	6	4	3	2	1	1
No. sperm/animal	10,000	5,000	5,000	10,000	10,000	10,000	10,000	10,000	10,000
Disomy XX	0.067	0.044	0.022						
Disomy YY	0.029	0.037	0.045	0.112	0.058	0.006	0.125	0.009	0.170
Disomy XY	0.029	0.031	0.012						
Total dis. (a)	**0.125**	**0.112**	**0.079**	**0.112**[a]	**0.058**[a]	**0.006**[a]	**0.125**[a]	**0.009**[a]	**0.170**[a]
Diploid XX	0.021	0.015	0.018						
Diploid YY	0.024	0.006	0.004						
Diploid XY	n.d.	0.029	0.041						
Total dip. (b)	**0.045**	**0.050**	**0.063**	**0.192**	**0.133**	**0.140**	**0.170**	**0.350**	**0.210**
(a) + (b)	0.170	0.162	0.142						
Reference[b]	(1)	(2)	(2)	(3)	(3)	(3)	(3)	(3)	(3)

[a] Data available only for YY disomy. [b] (1) Hassanane et al. [1999]; (2) present study; (3) Rubes et al. [1999].
S.F. = Swedish Friesian; I.F. = Italian Friesian; I.B. = Italian Brown; L.W. = Large White; B.F. = Bohemian Fleshy; L = Landrace; W.I. = White Improved; D. = Duroc; P. = Pietrain.

Table 4. Comparison of the disomy and diploidy frequencies using the two different sets of probes and disomy frequency for chromosome 6.

Breed	Sub.	Disomic XY		Diploid		Disomic 6
		probes X+Y	probes XY+6	probes X+Y	probes XY+6	
I.F.	1	0.117	0.134	0.017	0.022	0.038
	2	0.215	0.233	0.020	0.019	0.077
	3	0.175	0.157	0.038	0.029	0.098
I.B.	4	0.117	0.136	0.040	0.038	0.097
	5	0.058	0.058	0.057	0.030	0.116
	6	0.155	0.136	0.000	0.009	0.058
Total		0.139	0.142	0.029	0.025	0.080

X+Y = Xcen labeled in red; Y labeled in green.
XY+6 = Xcen and Y labeled in green; 6 labeled in red.